Pasting Cell: An Alternative Sample Cell for Detection of *Aspergillus flavus* Infected Milled Rice by NIR Spectroscopy

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ABSTRACT

The objective of this study was to reduce the quantity of milled rice sample used in spectra acquisition system for near infrared spectroscopy (NIRS). The milled rice (non-infected) and the milled rice mixing with Aspergillus flavus infected milled rice at the ratio of 5, 10, 15 and 20% w/w were investigated. The samples were contained in the coarse sample cell and were measured by the reflectance spectra using NIRSystem6500 in wavelength region from 700 to 2500 nm. Then, the same samples were packed in the pasting cell before measuring the spectra. The smoothing and second derivative techniques were used to transform the spectral data. The calibration equation was developed by partial least squares regression (PLSR). It was found that the value of the correlation coefficients (R) of the PLSR calibration result of the pasting cell were higher than the coarse sample cell, which were 0.97 and 0.82 respectively. Moreover, the standard errors of calibration (SEC), the standard errors of prediction (SEP), and the averages of difference between actual and NIR values (Bias) of the pasting cell were lower than the coarse sample cell. They were 1.60, 1.82, -0.28% and 4.04, 4.18, 0.02% respectively. Therefore, the pasting cell could be replaced by the coarse sample cell effectively, and the quantity of milled rice can be reduced.

Key words: Milled rice, Near infrared spectroscopy, *Aspergillus flavus*, Sample cell

INTRODUCTION

Aspergillus spp. and *Penicilluim* spp. were reported to be the major storage fungi of rice (Nguyen et al., 2007). *Aspergillus flavus* could produced mycotoxins which were well known for their health-hazardous effects in human beings and animals (Reiter et al., 2010), particularly aflatoxin B_1, which was moreover considered the main hepatocarcinogen (Speijers and Speijers, 2004). The fungal contamination of milled rice was investigated by Agar method or Blotter method, which is a high cost and consumes a lot of time. Moreover, these methods destroyed the sample. Near infrared spectroscopy (NIRS) is a low cost, rapid, repeatable, chemical free method and requires minimal or non sample preparation for the quality assessment. Moreover, many different constituents and properties of the sample could be analyzed at the same time (Fleurat-Lessard, 1997). It was a procedure for the detection of organic compounds. In the past, this technique was used to detect the fungal contamination of cereals and plants. For the example, Roberts et al. (1987) were able to quantify mold in alfalfa hay by NIRS determination of chitin and evaluated the calibration equation on a practical basis. Roberts et al. (1991) used NIRS to predict the fungal chitin content

in barley, the correlation of the chitin content and a visual estimation of the mold growth in the incubated sample was studied. Furthermore, Dowell et al. (1999) were able to predict the ergosterol content in wheat using NIR spectra from single a kernel and NIR has also been used to successfully predict fumonisin B$_1$ and ergosterol in maize (Berardo et al., 2005). Phetkaeo et al. (2011a) used VIS/NIRS to identify *A. flavus* and *A. niger* isolated from maize seed and used VIS/NIRS to detect *A. flavus* which contaminated maize seed (Phetkaeo et al., 2011b). Klaithin et al. (2011) used NIRS to detect *A. flavus* infected milled rice, but they used the coarse sample cell which required a bigger sample and took a long time for packing. Therefore, the objective of this study was to reduce the quantity of the milled rice sample used for detection of the *A. flavus* in infected milled rice. Two different sample cells were used for developing the calibration equation. The predicted value of the calibration equations and the actual value of the quantity of mixing milled rice infected with *A. flavus* were compared.

MATERIALS AND METHODS

Sample preparation

The milled rice cv. Khao Dawk Mali 105 was separated into two sets, which were the milled rice (non-infected) and the milled rice mixed with *A. flavus* infected milled rice. The surface of milled rice grain (non-infected) was disinfected by dipping in a 0.1% sodium hypochlorite solution for 1 min, rinsing in sterile water and drying on paper. The preparation of *A. flavus* infected milled rice, was done by inoculated rice sample with 1.4 ml of *A. flavus* spore suspension, its concentration was approximately 106 spores/ml. After that, it was plated on potato dextrose agar (PDA) and incubated at 37°C for 2 days. When the mycelial was present on the infected milled rice, it was mixed into the milled rice (non-infected) at the ratio of 0, 5, 10, 15 and 20% w/w. The number of 30 samples for each treatment were used. The samples were contained in the coarse sample cell (the sample weight ~ 200 g) (Figure 1A) and the reflectance spectra were measured using NIRSystem6500 (FOSS NIRSystem, Silver Spring, USA) in wavelength region 700 to 2500 nm. Then, the samples were packed in the pasting cell (the sample weight ~ 60 g) (Figure 1B) before measuring the spectra. The partial least squares regression (PLSR) was used to develop calibration equation.
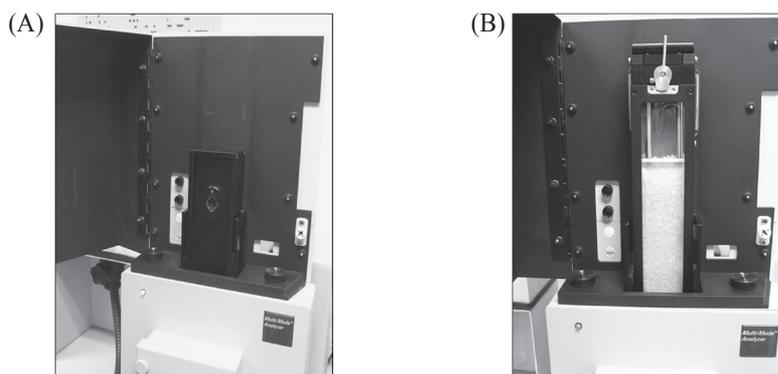

Figure 1. Two types of the sample cell, (A) the coarse sample cell and (B) the pasting cell, were used to contain the sample before measuring the spectra by NIRSystem6500.

RESULTS AND DISCUSSION

The means of the original spectra of the sample, the milled rice (non-infected) and the mixing milled rice with *A. flavus* infected milled rice at the ratio of 5, 10, 15 and 20% w/w in two sample cells, were shown in Figure 2. Three clear peaks at wavelength 1200, 1426 and 1904 nm were found in the means spectra of all samples packing in the coarse sample cell (Figure 2A) and

the peaks at 1208, 1426 and 2310 nm were found in the pasting cell (Figure 2B). Peaks at 1426 and 1904 nm correlated to O-H group of water molecule. Since the water is the main chemical component in agricultural products and it could well absorbed in NIR range. Then, the absorbtion of water was high and it could have overlapped the peak of the other molecules (e.g., starch) (Osborne et al., 1993). Peaks at 1200 and 1208 nm correlated to amino acids absorption band which were N-H group in molecules (Delwiche and Hareland, 2004). Meanwhile, peak at 2310 correlated to fat which was C-H group in molecule (Osborne et al., 1993).
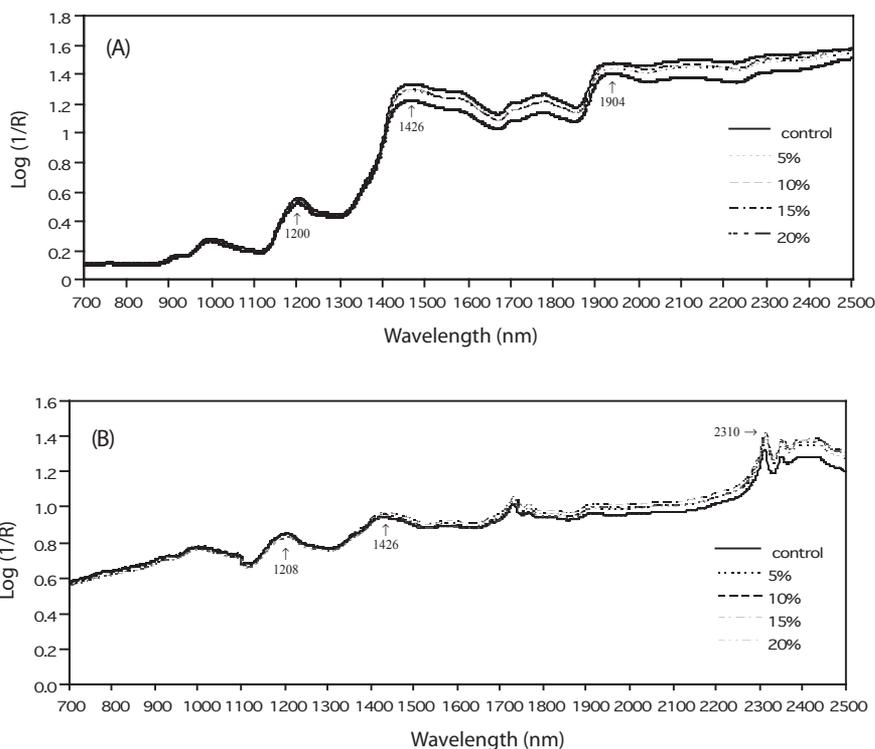

Figure 2. The means original spectra of milled rice (non-infected) and mixing milled rice were packed in the coarse sample cell (A) and the pasting cell (B).

The absorbance in whole wavelength (700 to 2500 nm) of the mixed milled rice (infected) were lower than the milled rice (non-infected). It was caused from light scattering which changed the light direction and increased pathlength. So, it affected the absorbance value of the mixtured sample at the different levels (Figure 2). *Aspergillus flavus* infected milled rice was easily broken. Their sizes were smaller than milled rice (non-infected). When the infected milled rice was mixed into the milled rice (non-infected) at the ratio of 5, 10, 15 and 20% w/w and they were packed in the sample cell, the light scatterings were lower than the milled rice (non-infected). Since, the small particle size would be tightly more compressed than the large particle size (Osborne et al., 1993).

The principle component analysis (PCA) was used to analyze the spectral data of the sample when packing in the coarse sample cell and the pasting cell (Figure 3). The number of independent variables, or variables derived from light absorption by NIRS can be reduced, which were related to create a new component, called the principal combination (PC) using the data of full spectrum. The spectra of milled rice (non-infected) and the mixture at 5% w/w packing in the coarse sample

cell could be separated with PC1 but the pasting cell was not (Figure 3). The mixture at 5% w/w was the lowest infection level for NIRS detection. So, the relation between the absorbance value and the chemical values were investigated.
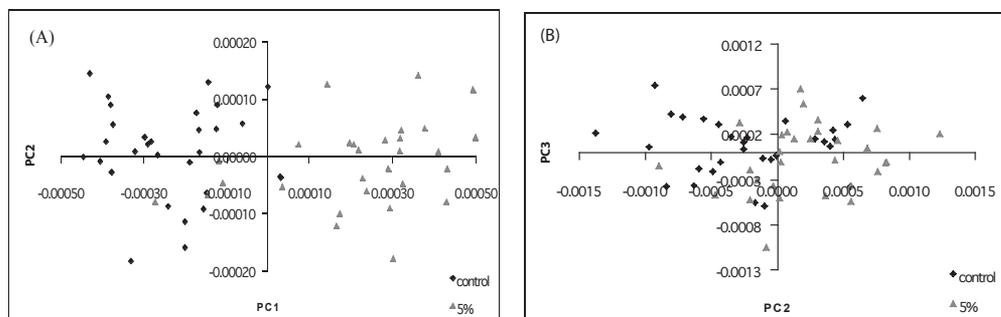

Figure 3. Principle component analysis plots of the spectra of milled rice (non-infected) and mixing of *Aspergillus flavus* infected milled rice at 5% w/w were contain in the coarse sample cell (A) and the pasting cell (B).

Two mathematical techniques, Savitzky-Golay smoothing (10 nm averaging for left and right side) and second derivative (5 nm averaging for left and right side) were used to transform the spectral data of the samples which were packed in the coarse sample cell and pasting cell. The spectral data were reduced the effects of base line shift and overlapping peaks by smoothing and second derivative. The calibration equation for detection of the levels of mixing infected milled rice was developed by using partial least square regression (PLSR). The calibration equation of the two samples cell (coarse sample cell and the pasting cell) were shown in Table 1.

Table 1. PLSR calibration equation for detection of *Aspergillus flavus* infected milled rice when packing in the coarse sample cell and the pasting cell.

Type of sample cell	Pre-treatment	Wavelength region (nm)	F	R	SEC	SEP	Bias
Coarse sample cell	Smoothing 10 + 2nd Derivative 5	1420-2468	4	0.82	4.04	4.18	0.02
Pasting cell	Smoothing 10 + 2nd Derivative 5	1130-2468	7	0.97	1.60	1.82	-0.28

F: latent variables or number of factors used in the calibration equation, R: multiple correlation coefficients, SEC: standard error of calibration, SEP: standard error of prediction, Bias: average of difference between actual value and NIR value.

The best PLSR calibration equation for prediction of the mixing level of infected milled rice in the coarse sample cell and the pasting cell were developed by using the spectral data in wavelength range 1420-2468 nm and 1130-2468 nm, respectively. The factors the calibration equation of samples packing in the pasting cell were bigger than of the coarse sample cell, there were 7 and 4, respectively. The correlation coefficient (R) and the average difference between actual value and NIR predicted value (Bias) of the pasting cell and the coarse sample cell were 0.97, -0.28% and 0.82, 0.02%, respectively. The standard errors of calibration (SEC) and the standard errors of prediction (SEP) of the pasting cell was lower than the coarse sample cell which were 1.60%, 1.82% and 4.04%, 4.18%, respectively (Table 1).

The regression coefficient plots showed the wavelengths which affect the PLSR calibration equation result. It means the wavelength or peak must be related to the interesting chemical component in the molecule. Various peaks at 1876, 2194 and 2362 nm were found in PLSR calibration

equation result of the coarse sample cell while peaks at 1400, 2186 and 2340 nm were found in the pasting cell (Figure 4). The results of Shenk et al. (2001) and Osborne et al. (1993) explained that peaks at 1410 and 1876 nm related to fat and starch. Their molecules obtained the chemical functional groups, C-H and O-H. Hecht and Wood (1956) found that wavelength at 2194 nm related to protein, the chemical functional groups are N-H and O=C-NH. Moreover, peaks at 2340 nm and 2356 nm related to chitin which is the main composition of mycelia in *A. flavus*. There are two the chemical function groups, C-N and N-H (Roberts et al., 1987).
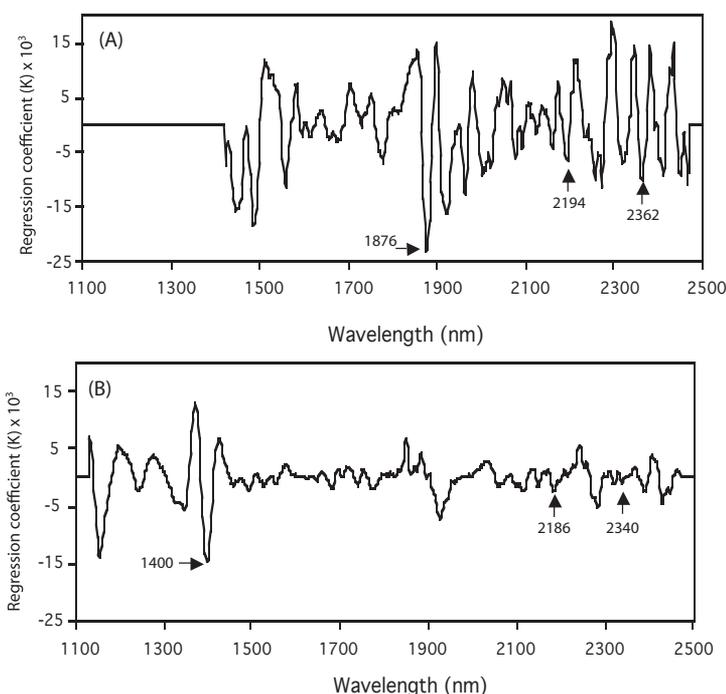

Figure 4. Regression coefficient plots of the calibration equation to predict the mixing level of *Aspergillus flavus* infected milled rice when packing in the coarse sample cell (A) and the pasting cell (B).

On the other hand, the peak of the coarse sample cell was clearer than peak of the pasting cell, but the result of the calibration equation in the pasting cell was better than the coarse sample cell.

Further studies would give more information on the extent to which a calibration can assist in avoiding grain with a susceptible high content of mycotoxins. Besides that, the *A. flavus* contamination of the lower level than 5% w/w should be further investigated.

CONCLUSION

The results indicated that the pasting cell could be used to peak the sample for NIRS detection of *A. flavus* infected milled rice in bulk grain samples and could replace the coarse sample cell effectively. It provided the better result than the coarse sample cell. Moreover, the quantity of milled rice in the samples was less than those of the coarse sample cell, which was not affected by the calibration substantially.

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